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NOTES FOR STUDENTS

Physiology of fungi.—The increasing attraction of this subject is evidenced by the number of papers that have appeared recently. The Graduate Laboratory of the Missouri Botanical Garden is publishing a series of such papers, 4 of which are noted herewith.

DUGGAR and DAVIS¹ deal with the often investigated problem of nitrogen fixation. Using a method by which the fungi were grown, digested, and distilled in the same flask without transfer, they were unable to demonstrate nitrogen fixation by Aspergillus niger, Macrosporium commune, Penicillium digitatum, P. expansum, and Glomerella Gossypii. In cultures of Phoma Betae on mangel and on sugar beet decoction with sugar, a nitrogen gain of 3.022-7.752 mg. was established, which they take to be a definite proof of fixation. A good review of the literature is included.

ZELLER² reports the following enzymes as occurring in a specially prepared enzyme powder from the wood destroying fungus *Lenzites saepiaria*: esterases, maltase, invertase, raffinase, emulsin, tarmase, diastase, inulanase, ligninase, cellulase, hemicellulase, pectinase, urease, hippuricase, nuclease, proteinase (both tryptic and ereptic), rennetase, oxidase, and catalase. Pectase and lactase were not demonstrated, and only slight indications were found of the presence of amidase. A comparative study of the enzymes occurring in sporophoral and mycelial tissue showed that the important metabolic processes are carried on in the latter.

ZELLER³ also deals with the physical properties of wood in relation to decay. On the basis of an extensive series of tests, he concludes, contrary to the opinion of other workers in this field, that resin is no safe index of the durability of the 3 species of yellow pine investigated. If it increases durability at all, it does so more by its waterproofing effect than by the toxic effect on the growth of fungi sometimes claimed for it. As a more reliable and practical

¹ DUGGAR, B. M., and DAVIS, A. R., Studies in the physiology of the fungi. I. Nitrogen fixation. Ann. Mo. Bot. Gard. 3:413-417. 1916.

² ZELLER, S. M., Studies in the physiology of the fungi. II. Lenzites saepiaria Fries., with special reference to enzyme activity. Ann. Mo. Bot. Gard. 3:439-512. 1916.



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criterion of durability, he recommends specific gravity, which, he says, is easily determined by inspection. The points to be noted are the proportion of summer wood to spring wood in the growth rings, and the width of the growth rings. If these are narrow, if the proportion of summer wood is high, and if the proportion of sap wood is low, the piece of pine can be considered of high specific gravity and therefore durable.

DUGGAR, SEVERY, and SCHMITZ⁴ have made a study of the growth of Macrosporium commune, Aspergillus niger, Glomerella (Gloeosporium) Gossypii, and Penicillium expansum on decoctions made from green string beans, corn meal, fresh turnips, sugar beets, dried prunes (exclusive of seed), and potatoes. Besides the natural decoctions, variants of these were used, containing, in addition to the plant extracts, different amounts of acid or alkali, cane sugar, potassium nitrate, and potassium acid phosphate. They found that the addition of sugar, nitrate, and phosphate gave in every case except one (Glomerella on bean decoction) increase in growth over the addition of sugar alone: Usually the next highest growth occurred when sugar and nitrate were added. Sugar alone gave a relatively slight increase over the natural decoction. The prune decoction seemed less favorable for growth than any of the others, except in the case of Macrosporium. Hydrogen-ion determinations, made by the colorimetric method, showed that in all solutions except the sugar beet and the corn meal decoctions Aspergillus caused a shift toward the acid side usually about 10⁻³, while Macrosporium and Glomerella generally caused a pronounced shift in the other direction. It is worthy of note here that REED⁵ found an increase in alkalinity in cultures of Glomerella rufomacullans, while the writer⁶ has shown the same condition to hold in case of apple bark attacked by blister canker (Nummularia discreta). Penicillium caused

an increase in acidity in the natural and standardized decoctions.

From the results of an investigation of the mosaic diseases of plants, FREIBERG⁷ comes to the conclusion that the infectious substance is an enzyme and not a virus, as ALLARD claims to have shown in recent work on the mosaic disease of tobacco. FREIBERG's reasons for his conclusion are that the infective principle is adsorbed by talc, and is destroyed by concentrations of alcohol and by temperatures which are destructive to enzymes. The fact that the infective principle is destroyed by formaldehyde is due, he thinks, to a specificity

⁴ DUGGAR, B. M., SEVERY, J. W., and SCHMITZ, H., Studies in the physiology of the fungi. IV. The growth of certain fungi in plant decoctions. Ann. Mo. Bot. Gard. 4:165-173. 1917.

⁵ REED, H. S., The enzyme activities involved in certain fruit diseases. Va. Exp. Sta. Rept. 1911–1912 (pp. 51–78).

⁶ ROSE, D. H., Oxidation in healthy and diseased apple bark. BOT. GAZ. 60:55-



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of reaction between the two and not to the antiseptic properties of the formaldehyde. This explanation he finds further supported by the fact that the infective power of extracts from diseased plants is not destroyed by treatment for two days with concentrated solutions of ether, chloroform, carbon tetrachloride, toluene, acetone, and glycerine. In this connection it is well to remember a statement by SMITH⁸ that in a number of organisms tested by him 10 grew in the presence of chloroform (5 cc. of chloroform in test tubes with 10 cc. of milk or beef bouillon), and 2 grew vigorously in the presence of thymol. He further states that, "in the opinion of the writer, statements of physiologists respecting the existence of enzymes in the tissues and fluids of higher plants must be taken with much allowance when chloroform, thymol, and similar antiseptics have been depended upon to keep the solution free from bacteria. A medium to which chloroform or thymol has been added must be shut in and shaken continuously if the full antiseptic value of these substances is to be obtained." Microchemical tests showed starch and sugar present in greater amounts in the dark green than in the chlorotic areas. FREIBERG suggests that this condition, taken in connection with the specificity of reaction between formaldehyde and the infective principle, and the possibility that formaldehyde is one of the first products of photosynthesis, may form a basis upon which the physiological nature of mosaic diseases may be explained. The possible relation of these factors to the formation of an enzyme is not made clear. Neither is it made clear how an enzyme can "reproduce itself." If it does so, why is not ALLARD'S contention the better one, that the causal agent is an organism and not an enzyme? And if it be granted for the sake of argument that the blotching of the leaves is caused by an enzyme, the question of the origin of the enzyme is still unanswered. In the work of ABERHALDEN and of KNUDSON, cited by FREIBERG, the development of proteolytic enzymes or of tannase was not spontaneous, but resulted from a stimulus foreign to the organism. Yellows or wilt, a serious disease of cabbage in many parts of the country, has recently been investigated by Gillman.⁹ He finds that the causal fungus, Fusarium conglutinans Wollenw., has a high optimum temperature and is very resistant to drying, both in pure culture and in the soil. The characteristic symptoms of the diseases are dependent on a temperature of about 17-22° C. or above for their occurrence. Lower temperatures (12-16° C.) under controlled conditions prevented the occurrence of the trouble in the greenhouse.

Two rusts of economic importance, Puccinia coronata Cda. and P. Sorghi Schw., are the subject of a physiological investigation by MAINS.¹⁰ The

⁸ SMITH, E. F., Bacteria in relation to plant diseases. Ann. Mo. Bot. Gard. 1:74, 75. 1905.

⁹ GILLMAN, JOSEPH C., Cabbage yellows and the relation of temperature to its occurrence. Ann. Mo. Bot. Gard. 3:25-84. 1916.

¹⁰ MAINS, E. B., The relation of some rusts to the physiology of their hosts. Amer. Jour. Botany 4:179-221. 1917.

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optimum temperature for the former is put at about 20° C., for the latter 30°. The fact that no injury appears in the infected cells, but only in the cells surrounding them, is thought by MAINS to be due to starvation brought about by withdrawal of foods to the infected region. It is possible, however, that the injury might be caused by injurious enzymes or other toxic substances which diffuse outward from the infected cells. The growth of the rusts and the development of spore pustules were increased when some carbohydrate was added to the nutrient solution, and the conclusion is drawn that "the obligate parasitism of the rusts is probably explained by their requirement of some transitory or nascent organic products related to the carbohydrates which they obtain in the living plant." This conclusion is hardly in accord with the statement made by RUSSELL^{II} that wheat plants whose photosynthetic activity has been seriously decreased by lack of potash, and whose carbohydrate content is therefore low, are especially susceptible to attacks of rust. Further work seems necessary to clear up the situation. BROOKS and COOLEY¹² find that in inoculations on apples all of the fungi tested grew at o° C. except Fusarium radicicola and Glomerella cingulata, the former making no growth at 15° and the latter none at 10°. Sphaeropsis malorum had produced no evident rot at 15° by the end of a week, the species of Penicillium and Neofabraea at 10° by the end of two weeks, while Sclerotinia cinerea produced measurable rots at 5° in one week and at o° in two weeks. Neofabraea malicorticis had an optimum at 20°, Fusarium radicicola at 30°, and all the other fungi at 25°. When grown on corn meal agar in Petri dishes, all the fungi showed the same optimum and maximum as in the fruit inoculation experiments. With most of the fungi the initial incubational stages of growth on the fruit were more inhibited by low temperatures than the later

ones. The results of the investigation show the importance of immediate as compared with delayed storage; the value of temperatures of 5 or 10° in short periods of storage, and of 0° in longer ones; and further that the minimum temperature varies with the prevalent fungus and with the variety and maturity of the fruit.

In an investigation of the growth of fungi on nutrient solutions by HAWKINS¹³ it was found that *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea* grew readily in solutions of potassium and calcium nitrate, sucrose, and glucose in which the diffusion tensions were much higher than the total diffusion tensions of the dissolved substances in the juices of their host plants.—D. H. ROSE.

¹¹ RUSSELL, E. J., Soil conditions and plant growth. 2d ed. London. 1915 (pp. 41, 42).

¹² BROOKS, CHARLES, and COOLEY, J. S., The temperature relations of apple-rot

